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(71) Applicant *(for all designated States except US)*: FORBIO RESEARCH PTY. LTD. [AU/AU]; 50 Meiers Road, Indooroopilly, QLD 4068 (AU).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: TEASDALE, Robert, Dixon [AU/AU]; 33 Maculata Drive, Chapel Hill, QLD 4069 (AU).

(74) Agent: PIZZEYS PATENT AND TRADE MARK ATTORNEYS; Level 6, 444 Queen Street, Brisbane, QLD 4000 (AU).

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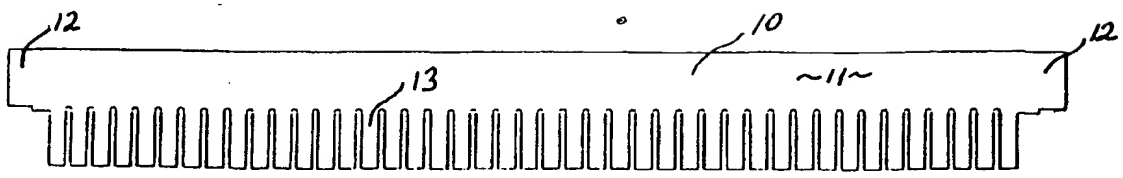

(57) Abstract

There is provided an electrophoresis method including casting wells in a gel with a gel tooth comb (10) having a body portion (11) extending between supporting lugs (12) and a plurality of gel teeth (13) comprising a lower portion (14) corresponding to the substantive well portion, and an upper portion (15), the transition in thickness from the lower tooth portion (14) to the upper tooth portion (15) being provided by a 45° sloping portion (16) disposed in the direction away from the electrophoresis direction.

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ELECTROPHORETIC METHOD AND APPARATUS

This invention relates to an electrophoretic method and apparatus therefor.

This invention has particular but not exclusive application to methods and apparatus for use in gel electrophoresis of DNA and RNA samples, and for illustrative purposes reference will be made to such application. However, it is to be understood that this invention could be used in other applications, such as electrophoretic determinations of proteins and other charged molecules.

Comparative electrophoresis requires that multiple lanes of a gel are loaded with samples which are then run under the same condition variables to provide a calibrated, qualitative or quantitative result comparing respective lanes. A limitation on the experimental and productive use of electrophoretic methods has been limitations on available resolution of electrophoresis using automated loading processes.

In order to automate electrophoretic analysis, or to run high-multiple gels, gel combs are currently used. Gel combs are toothed forms, usually of TEFLON or other polymer, which are positioned in a tray into which an agarose or other gel material is cast, so that after the gel has set, the comb can be removed to leave a set of cavities, or wells, corresponding to the shape and position of the teeth on the comb. Sample solutions are then placed in these wells using a pipette dispenser, after which the sample components are separated by electrophoresis.

This process is often assisted by increasing the density of the sample solution so that it is higher than a "running buffer" with which the wells are normally filled. This general arrangement is used for separation of biological macromolecules such as DNA, RNA, enzymes and other proteins, and some carbohydrates, among others.

Where separations of high resolution are required, the thickness of the well can be a limiting factor. However, as the well becomes thinner, the loading of sample is correspondingly more difficult, requiring precise positioning of the pipette tip into the well. This task is more difficult when loading occurs with a multi-channel pipettor, such as various commonly used octi-pipettes, where any slight deflection or rotation of the comb or the octi-pipette can result in the misloading of some samples.

Similarly most laboratory robots for automated loading, using either single or multi-channel systems, are unable to reliably dispense into thin wells, particularly when deflections in combs or variations in positioning of tips on robotic tools are similar to the well thickness. The loss in resolution through use of suitably thick combs can offset the benefits obtained through more efficient loading.

One solution proposed to this problem was the removal of wells altogether through use of gel end-plates which close off the end of a tray during gel casting. The gel end plates have small depressions which are machined corresponding to each sample lane. Sample solutions were individually mixed with a hot agarose solution and, using a simple laboratory robot,

these solutions then readily pipetted into the machined depressions with the end-plate laid flat. After the sample aliquots have set in these depressions, the end-plate is then placed in the gel tray in its normal orientation. Hot agar is then poured into the gel tray, as for other situations. When the gel has set the samples are then "fused" to the outside of the gel, and can be electrophoresed into the gel.

However, this system suffers from several problems. First, the mobility of the sample components will be reduced in dilute agar compared to free solution in normal wells. As the sample aliquot has some thickness, sample resolution is therefore already diminished in comparison with wells of similar thickness. Secondly, the heat present during casting of the main gel results in diffusion of the sample. Thirdly, evaporation from the sample gel tends to concentrate salts in the gel which can lead to variation in mobility of the sample components, and uneven sample evaporation will lead to distortion of component mobilities. Fourthly, surface tension effects in the sample gel will lead to a non-linear sample front.

In general, these difficulties have been tolerated when they are outweighed by the benefits of automation. Where automation is not possible because of these limitations, then standard labour intensive procedures have had to have been used. To date no automated high resolution processes have been available.

The present invention aims to substantially alleviate at least one of the above disadvantages and to provide an

electrophoretic method and apparatus which will be reliable and efficient in use. Other objects and advantages of this invention will hereinafter become apparent.

With the foregoing and other objects in view, this invention resides broadly in an electrophoretic method including providing a gel having a plurality of sample wells integrally formed in the gel, said sample wells each having a substantially flat front face of width sufficient to provide an appropriate lane width, and a lower rear face spaced apart therefrom by a sample width selected to provide a selected resolution, and an upper rear face diverging from said front wall to provide a loading opening for said well larger than said sample width to facilitate automated loading of said wells.

The wells may be formed by any suitable means and are preferably formed by a gel comb.

Accordingly, in a further aspect this invention resides in a gel comb comprising a body member adapted to support a plurality of spaced well forming members substantially vertically in a gel to be cast, each well forming member having a substantially flat front surface of width sufficient to provide an selected lane width, and a lower rear surface spaced apart therefrom by a dimension selected to form sample well of selected resolution by width, and an upper rear face diverging from said front surface.

The lower portion of the gel comb well forming members includes a lower rear surface, the thickness of the well forming member being selected to provide wells having the

appropriate dimension in the direction of electrophoresis to provide a selected resolution in the gel to be cast.

Of course, the exact thickness of the well forming member at this point will be determined by the samples to run, the gel type, and many other variables. In general, the thickness of the well forming member at this point will be of the order of the thickness of a high resolution, manually loaded well. For DNA and RNA determinations, the thickness may be in the region of 0.5mm, which is generally regarded as too small for automated loading.

The vertical length of the well forming member lower portion will be selected to provide an appropriate sample volume for a selected gel thickness. For nucleic acid samples, where the aforementioned 0.5mm well thickness is selected, wells formed are in the region of 3.3mm wide and 3.5mm high to the filling region.

The upper portion of the well forming member includes an upper rear surface portion extending upward and away from the front surface at about 45°. By this means, the resulting well may present an upper opening that is of a plan dimension adequate for use of automated and multiple loading such as by means of an octipipette. The divergent upper rear surface portion is preferably adapted to be inserted into the gel until the opening at the surface is just sufficient for gel loading, although it is envisaged that the well forming member may be inserted further into the gel since the filling height is determined by the dispensed volume.

Preferably methods in accordance with the present

invention include forming the wells in the gel, filling the wells with a displaceable buffer or other liquid and loading the samples in dilute gel into the wells, whereby the buffer or liquid is displaced from the wells. Gel loaded samples are preferably loaded such that the sample meniscus is located at the lower region of the divergent portion of the well. Whilst precision loading of narrow wells in the past has proved problematical, the wells of the present invention include an upper divergent portion which also acts as a funnel so that the entire sample aliquot flows into the narrow part of the well.

The well forming members are preferably integrally formed with the body member of suitable material to produce a gel tooth comb. The spacing of the gel teeth may be selected to be any suitable spacing and can be such as to correspond to standard distances encountered in multi-channel dispensers. For example the mean spacing of teeth may be 4.5mm, which is twice the separation of tips in standard octi-pipette dispensers, which in turn corresponds to the 9mm separation of wells in standard 96-well multi-well trays for many biochemical and molecular-biological reactions.

When samples originate in multi-well trays, each second well formed in the gel may correspond to a row of wells in one tray, with the intermediate gel samples corresponding to the second row; with appropriate placement of samples in the sample tray any required order of loading on gels can be attained. The use of wider teeth to match precisely the spacing of multi-tip dispensers is also envisaged. The preferred thickness of the teeth of 0.5mm provides excellent resolution for DNA

samples in the range 400 to 15000bp. Thinner teeth can provide narrower bands, but a limit to improved resolution is reached due to diffusion, so that thinner bands will lead to lower signals and signal to noise ratios. With gels of low density, some distortion of well walls can lead to actual well thickness being less than that of the comb, and optima are best empirically determined for particular systems. The combs can be constructed of any machinable or castable material compatible with the gel buffers, and may include brass, stainless steel, TEFLON, or other polymers.

In order that this invention may be more readily understood and put into practical effect, reference will now be made to the accompanying drawing and following examples which illustrate a preferred embodiment of the invention and wherein:

FIG. 1 is a front elevation of apparatus in accordance with the present invention, and

FIG.2 is an end elevation of the apparatus of FIG 1.

In the Figures, there is provided a gel tooth comb 10 having a brass body portion 11 extending between supporting lugs 12 of standard dimensions. Formed integrally with the body portion 11 are a plurality of gel teeth 13, in this example of mean spacing 4.5mm, which is twice the separation of tips in standard octi-pipette dispensers, which in turn corresponds to the 9mm separation of wells in standard 96-well multi-well trays for many biochemical and molecular-biological reactions. When samples originate in multi-well trays, each second well formed in the gel corresponds to a row of wells in one tray, with the intermediate gel samples corresponding to

the second row; with appropriate placement of samples in the sample tray any required order of loading on gels can be attained.

The teeth 13 comprise a lower portion 14 corresponding to the substantive well portion, and an upper portion 15. The lower portion in this example of the improved comb is 0.5mm thick, which provides excellent resolution for DNA samples in the range 400 to 15000bp. The lower portion 14 is 3.5mm long and 3.3mm wide. The upper portion 15 is 3 mm thick. The transition in thickness from the lower tooth portion 14 to the upper tooth portion 15 is provided by a sloping portion 16 disposed to the back of the comb, that is, in the direction away from the electrophoresis direction. The sloping portion 16 is disposed at 45° to the horizontal plane.

The use of the foregoing comb is described in the following example including comparison with standard combs.

EXAMPLE 1

The following example was constructed to demonstrate that the apparatus and method of the present invention gave better resolution images of RAPD fragment separation on agarose gels when compared to standard gel combs.

RAPD reactions were set up in a double volume, to enable the same reaction mix to be loaded on gels using the wedge comb and conventional comb. The four primers used were chosen for high levels of polymorphism. The primers were run on DNA samples from P. radiata and E. globulus. All RAPD fragments were separated using agarose gel electrophoresis using 1.5% gels run at 180V in 1 X TAE buffer for 2.5 hours.

The comparison was made by finding the resolutions of gels using both combs. Resolution of a gel is defined as the minimum band separation between two RAPD fragments from the same lane detectable by the naked eye. This is expressed as a percentage of the band size.

Where band1 and band2 are the closest separated bands in the gel and that band1 has a smaller molecular weight than band2. Testing data is as following:

Table 1 Primer L12 Resolution

	Standard Comb	Wedge Comb
band1 (bp)	1649	1225
band2 (bp)	1689	1243
size(band2)-size(band1)	40	18
resolution (%)	2.4	1.5

Table 2 Primer Y07 Resolution

	Standard Comb	Wedge Comb
band1 (bp)	880	872
band2 (bp)	904	889
size(band2)-size(band1)	24	17
resolution (%)	2.7	1.9

Table 3 Primer AL04 Resolution

	Standard Comb	Wedge Comb
band1 (bp)	928	781
band2 (bp)	949	793
size(band2)-size(band1)	21	12
resolution (%)	2.2	1.5

Table 4 Primer P11 Resolution

	Standard Comb	Wedge Comb
band1 (bp)	1727	1407
band2 (bp)	1775	1430
size(band2)-size(band1)	48	23
resolution (%)	2.7	1.6

Table 5 Resolution Comparison

Primer	Standard Comb Res. (%)	Wedge Comb Res. (%)	Res. Ratio
L12	2.4	1.5	1.60
Y07	2.7	1.9	1.42
AL04	2.2	1.5	1.47
P11	2.7	1.6	1.68
average	2.50	1.63	1.55

On average the resolution for wedge combs is 1.55 times better than the standard combs. Furthermore, there is a notable difference of the signal to noise ratio between the standard comb and the wedge comb. Signal to Noise ratio indicates the difference between band intensity and the background noise. That is the higher the ratio the brighter the

bands are in comparison to the background. Thus the bands are able to be distinguished from each other. The corresponding data for the above gels are as following:

Table 6 Wedge Comb Vs Standard Comb Resolution summary

Primer	Standard (Signal/Noise)	Wedge (Signal/Noise)	Wedge : Standard
Y07	4.96	8.54	1.72
L12	6.50	20.99	3.23
AL04	2.45	29.32	11.95
P11	5.91	7.13	1.21
average	4.95	16.50	4.53

In summary the signal to noise ratio increases by an average of 4.5 times when using the wedge comb instead of the Standard comb. It is also found that band widths have decreased using Wedge comb. Table 7 shows Wedge comb peak widths are 79% of the Standard comb peak widths. This decrease in peak width enhances the ability of bands to be detected.

Table 7 Wedge peak width vs Standard peak width

	Wedge peak width : Standard peak width (%)
Q9 Lane5	80.09
Q9 Lane7	79.81
L12 Lane2	75.26
L12 Lane5	81.61
average	79.19

In terms of data yield, using the gel scoring program -

Gel Pro Analyser, wedge comb gels tended to have a higher number of bands detected than the standard comb gels. Table 8 shows the corresponding number of bands in different gels of 4 primers.

Table 8 Number of detectable bands by Gel Pro

Primer	Standard Comb	Wedge Comb	Difference
YO7	41	51	10
P11	36	43	7
3.05	29	35	6
MO4	34	48	14
average	35	44.25	9.25

As a result wedge comb produces 26.4% more detectable bands on average.

In order to overcome the conflicting needs of increased information yield through enhanced resolution per separation and that through efficiency of loading, a simple gel comb has been designed which improves both resolution and ease of loading. The comb is particularly suited to automated robotic loading, but is also advantageous for manual loading by either single tip or multi-tip dispensers.

It will of course be realised that while the above has been given by way of illustrative example of this invention, all such and other modifications and variations thereto as would be apparent to persons skilled in the art are deemed to fall within the broad scope and ambit of this invention as defined in the claims appended hereto.

CLAIMS

1. An electrophoretic method including providing a gel having a plurality of sample wells integrally formed in the gel, said sample wells each having a substantially flat front face of width sufficient to provide an appropriate lane width, and a lower rear face spaced apart therefrom by a sample width selected to provide a selected resolution, and an upper rear face diverging from said front wall to provide a loading opening for said well larger than said sample width to facilitate automated loading of said wells.

2. An electrophoretic method according to Claim 1, wherein said plurality of sample wells are integrally formed in the gel by means of a gel comb.

3. An electrophoretic method according to Claim 2, wherein said wells are formed in the gel, the wells are filled with a displaceable buffer or other liquid and loading the samples in dilute gel into the wells, whereby the buffer or liquid is displaced from the wells.

4. An electrophoretic method according to Claim 3, wherein said loaded samples are loaded such that the sample meniscus is located at the lower region of the divergent upper rear face of the well.

5. An electrophoretic method according to any one of the

preceding Claims, wherein the sample is a nucleic acid and said sample width is selected to be in the region of 0.5 mm.

6. An electrophoretic method according to any one of the preceding Claims wherein said plurality of wells are spaced at a pitch of 4.5mm.

7. A gel comb comprising a body member adapted to support a plurality of spaced well forming members substantially vertically in a gel to be cast, each well forming member having a substantially flat front surface of width sufficient to provide an selected lane width, and a lower rear surface spaced apart therefrom by a dimension selected to form sample well of selected resolution by width, and an upper rear surface diverging from said front surface.

8. A gel comb according to Claim 7, wherein said front surface and said lower rear surface are spaced apart by about 0.5mm.

9. A gel comb according to Claim 8, wherein said upper rear surface diverges from said front surface at an angle of about 45°.

10. A gel comb according to Claim 9, wherein said lower rear surface has a height in the region of 3.5mm.

11. A gel comb according to any one of the preceding Claims 7

to 10, wherein said plurality of well forming members are spaced on said body member at a pitch of 4.5mm.

12. A gel comb according to Claim 11, wherein said well forming members are separated by spaces of 1.2mm.

13. A gel comb according to Claim 12, wherein the body member and well forming members are integrally formed from a material selected from a machinable or castable material compatible with gel buffers.

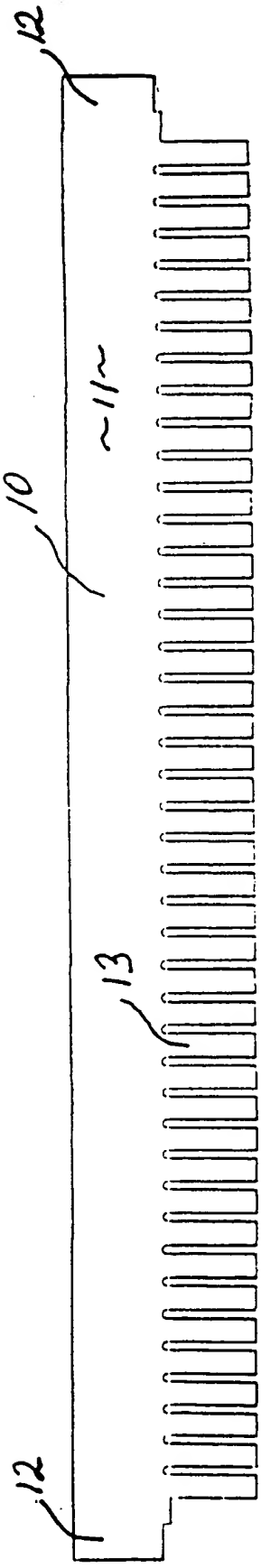

Fig 1

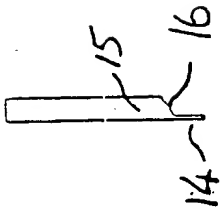

Fig 2

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 97/00828

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: G01N 27/447; B01D 57/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC[6]: G01N 57/447; B01D 57/02

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT, CHEM ABS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GB,A, 2284484 (UNIVERSITY COLLEGE LONDON) 7 June 1995	
A	US,A, 5318682 (SINGER) 7 June 1994	
A	US,A, 5284565 (CHU et al) 8 February 1994	
A	US,A, 5164065 (BETTENCOURT et al) 17 November 1992	
A	US,A, 4294684 (SERWER) 13 October 1981	
A	DD,A, 251829 (AKADEMIE DER WISSENSCHAFTEN) 25 November 1987	

☐ Further documents are listed in the continuation of Box C

☒ See patent family annex

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Date of the actual completion of the international search	Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

Information on patent family members

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Patent Document Cited in Search Report		Patent Family Member					
GB	2284484	WO	96/18891				
US	5318682	NONE					
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		GB	2273783				
US	5164065	NONE					
US	4294684	NONE					
DD	251829	DD	217275				

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